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EFFECT OF VARIATIONS IN OXYGEN TENSION ON THE TOXICITY OF SODIUM CHLORIDE ISOTONIC TO SEA WATER.

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Loeb first observed that he could protect fertilized *Arbacia* eggs for some time from the toxic action of a NaCl solution, isotonic to sea water, by removing the oxygen from the solution or by adding a little KCN.[1] Cyanide also protects unfertilized *Arbacia* eggs against injury by isotonic solutions of various sodium salts, and anæsthetics in appropriate concentrations have a similar effect.[2] Experiments with *Arenicola* larvæ have given similar process being in some manner connected with the destructive results.[3] These results indicate the probability of an oxidative process being in some manner connected with the destructive reaction.

The following experiments were undertaken to determine the effect of varying the oxygen tension on the toxicity of NaCl isotonic to sea water. The form chosen to work with was the larva of the marine worm, *Arenicola cristata,* a free swimming trochophore of three body segments, about ⅓ mm. in length. When normal it is almost constantly in motion, showing ciliary action and bendings to one side, or strong muscular contractions. The larvæ employed were all in the swarming stage and were collected from the lighted side of the culture dish, just below the surface of the water, where they gather in large numbers. As they remain in this stage only from three to four days before developing another segment and sinking to the bottom, a fairly homogeneous culture is obtained. When these animals are placed in pure sodium chloride isotonic to sea water they promptly con-

[1] Loeb, J., *Science,* 1910, XXXII., 411; *Biochemical Zeitschrift,* 1910, XXIX., p. 80.

[2] Lillie R. S., *Amer. Journ. Physiol.,* 1912, XXX., p. 1.

[3] Lillie, R. S., *Amer. Journ. Physiol.,* 1912, XXIX., p. 372, and 1913, XXXI., p. 255.

tract to about ⅔ of their former length and all motion ceases. This contraction is followed by a slow relaxation and eventually they are killed. But if replaced in sea water before death, they slowly recover either completely or to a certain extent. The ratio of those in motion to those still gives an index of the extent of the injury.[4]

TECHNIQUE.

The larvæ being positively heliotropic, gather in great numbers in the lighted side of the culture dish. A pipette-full (over 500 larvæ) was taken and placed in a watch glass, then, as the larvæ collected again on the light side, the water could be tilted off, the last traces being removed by blotting paper. They were next washed twice with the solution to be experimented with and then transferred by a pipette to 50 c.c. of that solution. Then at hourly intervals about 100 larvæ were removed from the test solution, washed twice with sea water and replaced in sea water. These were inspected one hour and in most cases also twelve hours after their return to sea water. Fifty individuals were examined and the ratio of those in motion to those still was determined.

EXPERIMENTAL.

I. The larvæ were first exposed to 0.52 molecular NaCl in almost complete absence of oxygen. The solution was boiled while a stream of hydrogen was passed through, care being taken

TABLE I.

EXPERIMENT 1.

Over 50 larvæ examined in each case.

Examined 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.	
	3 Hours.	6 Hours.
Isotonic NaCl at atmospheric oxygen tension	No motion	No motion
Isotonic NaCl at reduced oxygen tension	All larvæ show bendings	No motion

[4] For a further description of the behaviour of *Arenicola* larvæ in isotonic NaCl see Lillie, R. S., *Am. Jour. Physiol.*, 1909, XXIV., p. 14, and *Am. Jour. Physiol.*, 1911, XXVIII., p. 210.

to maintain the proper concentration and reduce the temperature to room temperature before introducing the larvæ. This procedure removes the oxygen almost entirely, for if alkaline pyrogallol is added to the solution after this treatment no color appears for an hour or more. The control solution, 0.52 NaCl at atmospheric oxygen tension, was also boiled, air instead of hydrogen being bubbled through.

TABLE II.

EXPERIMENT 1.

Over 50 larvæ examined in each case.

A. Examined 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.		
	1 Hour.	2 Hours.	3 Hours.
Isotonic NaCl at atmospheric oxygen tension.....	½ show feeble bendings, ½ show no motion	No motion	No motion
Isotonic NaCl at reduced oxygen tension.....	⅔ show bendings more violent than control	⅓ show bendings	No motion

B. Same Larvæ Examined 24 Hours after Return to Sea Water.

Isotonic NaCl at atmospheric oxygen tension.....	1 larva showed bendings. The rest had disintegrated.	All disintegrated	All disintegrated
Isotonic NaCl at reduced oxygen tension.....	All larvæ actively motile	All disintegrated	All disintegrated

The antitoxic effect of the absence of oxygen is marked. This was to be expected from Loeb's experiments on *Arbacia* eggs.

II. To expose the larvæ to 0.52 mm. NaCl with increased oxygen tension, the simple apparatus pictured was employed. The oxygen tension desired was obtained by running washed oxygen from a tank into the chamber "A," driving the level of water inside the chamber down to a mark previously determined

by calculating the volume required with the necessary corrections. The air already present in the chamber is thus diluted with pure oxygen to the required degree. The water levels in and out of the chamber were kept equal by removing water from the large jar "F." The apparatus is only approximately accurate.

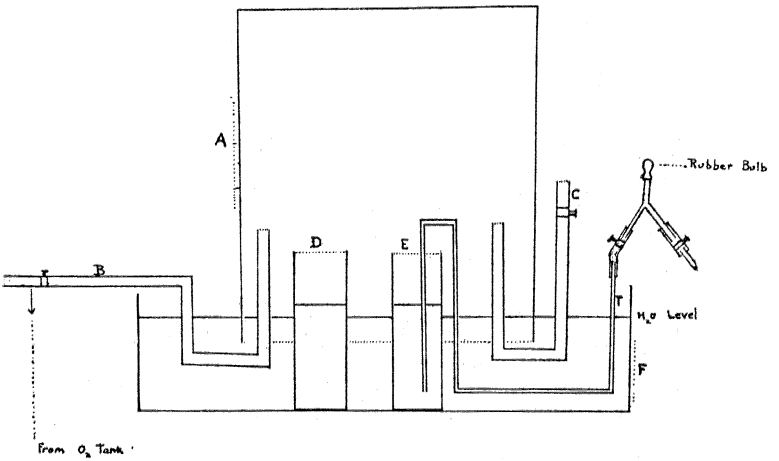

Diagram of apparatus.

The larvæ were introduced and removed by the tube "*T*" of 1 mm. bore, which was connected to a Y tube and the rubber bulb "*H*." By manipulating the rubber bulb and the two pinch cocks on the connections of the Y tube, it was possible to pump the larvæ in or out of the small beaker "*E*." This beaker contained the isotonic NaCl. A similar beaker "*D*" contained a control culture in sea water at the same oxygen tension as "*E*." A third beaker not shown in the diagram contained another control culture in isotonic NaCl at atmospheric oxygen tension.

In the first series of experiments the oxygen tension of the NaCl solution containing the larvæ was raised gradually. The larvæ were placed in the isotonic NaCl solution in the beaker "*E*" before the oxygen tension was raised in the chamber. The tension in the chamber was then raised to 320 mm. and the salt solution and control were slowly stirred for two minutes. The oxygen tension of the solution and control must rise slowly towards 320 mm., but how long it is before it reaches that figure

is difficult to predict. As it was impossible to stir too vigorously for fear of injuring the larvæ and as diffusion is slow across the surface of an unagitated liquid, it seems probable that the oxygen tension of the NaCl solution and the sea water in the control never reached 320 mm. during the experiment.

TABLE III.

EXPERIMENT 2.

Over 50 larvæ examined in each case.

A. Examined 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.		
	1 Hour.	2 Hours.	3 Hours.
Isotonic NaCl at atmospheric oxygen tension.....	All larvæ show bendings	No motion	No motion
Isotonic NaCl at slightly increased oxygen tension...	All larvæ show bendings	1/2 larvæ show bendings	1/4 larvæ show bendings
Sea water at slightly increased oxygen tension.....	Larvæ normal	Normal	Normal

B. Examined 21 Hours after Return to Sea Water.

Isotonic NaCl at atmospheric oxygen tension.....	No motion	No motion	No motion
Isotonic NaCl at slightly increased oxygen tension...	Larvæ completely recovered	1/10 larvæ show bendings	Occasional larva shows bendings
Sea water at slightly increased oxygen tension.....	Normal	Normal	Normal

TABLE IV.

EXPERIMENT 2.

Over 50 larvæ examined in each case.

A. Examined 1 Hour after Return to Sea Water.

Solution,	Time of Exposure.		
	1 Hour.	2 Hours.	3 Hours.
Isotonic NaCl at atmospheric oxygen tension.....	1/2 larvæ show bendings	No motion	No motion
Isotonic NaCl at slightly increased oxygen tension...	All larvæ show bendings	1/20 larvæ show bendings	1/10 larvæ show bendings
Sea water at slightly increased oxygen tension.....	Larvæ normal	Normal	Normal

B. Same Larvæ Examined 12 Hours after Return to Sea Water.

Isotonic NaCl at atmospheric oxygen tension.....	1/50 larvæ show bendings	No motion	No motion
Isotonic NaCl at slightly increased oxygen tension...	1/4 larvæ show bendings	1/12 larvæ show bendings	1/8 larvæ show bendings
Sea water at slightly increased oxygen tension.....	Normal	Normal	Normal

The toxicity is certainly markedly diminished by the slight increase in oxygen tension. The effect is as marked as when the tension was decreased.

III. In this experiment the oxygen tension of the chamber was raised to 230 mm. four hours before the experiment was started. The isotonic NaCl in the beaker "*E*" was stirred vigorously at intervals of fifteen minutes allowing the solution to come to equilibrium at an oxygen tension of 230 mm. before the larvæ were introduced. The larvæ were washed twice with this NaCl, at 230 mm. oxygen tension, by pumping a few c.c. out through the tube "*T*" and they were then introduced into the beaker "*E*" as

before. The larvæ were therefore suddenly subjected to the change of oxygen tension plus the NaCl.

TABLE V.

EXPERIMENT 3.

Over 50 larvæ examined in each case.

A. Observed 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.			
	1 Hour.	2 Hours.	3 Hours.	4 Hours.
Isotonic NaCl at atmospheric oxygen tension 158 mm.....	1/10 larvæ show bendings	1/5 larvæ show bendings	1/5 larvæ show bendings	No motion
Isotonic NaCl at approx. 230 mm. oxygen tension.....	All larvæ show bendings	9/10 larvæ show bendings	1/2 larvæ show bendings	1/50 larvæ show bendings
Sea water at approx. 230 mm. oxygen tension.	Larvæ normal	Normal	Normal	Normal

B. Observed 18 Hours after Return to Sea Water.

Isotonic NaCl at 158 mm. oxygen tension....	1/7 larvæ show bendings	1/100 larvæ show bendings	1/50 larvæ show bendings	1/100 larvæ show bendings
Isotonic NaCl at approx. 230 mm. oxygen tension.....	9/10 larvæ show bendings	1/2 larvæ show bendings	3/50 larvæ show bendings	2/50 larvæ show bendings
Sea water at approx. 230 mm. oxygen tension.	Normal	Normal	Normal	Normal

This experiment shows the same diminution of toxicity due to slightly increased oxygen tension.

IV. In this experiment the oxygen tension was raised to 275 mm. before the larvæ were introduced, as in the preceding experiment.

TABLE VI.

EXPERIMENT 4.

Over 50 larvæ examined in each case.

A. Observed 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.		
	1 Hour.	2 Hours.	3 Hours.
Isotonic NaCl at atmospheric oxygen tension 160 mm..	1/4 larvæ show bendings	1/50 larvæ show bendings	No motion
Isotonic NaCl at approx. 275 mm. oxygen tension...	1/4 larvæ show bendings	1/100 larvæ show bendings	No motion
Sea water at approx. 275 mm. oxygen tension.....	Normal	Normal	Normal

B. When observed 24 hours after return to sea water there was no motion in any case except the control in sea water at 275 mm. which was normal.

The parallelism with the control is striking. The toxic and antitoxic effects of the increase of oxygen tension must balance at this tension.

V. In the last experiment the larvæ were exposed to an oxygen tension of 756 mm. (saturated). A stream of oxygen was bubbled through a flask containing the isotonic NaCl for fifteen minutes. The larvæ were washed twice with this solution, then were placed in it and oxygen bubbled through for five minutes more and the flask closed. When some of the larvæ were removed at hourly intervals for return to sea water, oxygen was bubbled through the remaining solution for one minute immediately after.

TABLE VII

EXPERIMENT 5.

Over 50 larvæ examined in each case.

A. Observed 1 Hour after Return to Sea Water.

Solution.	Time of Exposure.		
	1 Hour.	2 Hours.	3 Hours.
Isotonic NaCl at atmospheric oxygen tension 160 mm..	3/4 larvæ show bendings	No motion	1/20 larvæ show bendings

Isotonic NaCl at approx. 756 mm. oxygen tension...	No motion	No motion	No motion
Sea water at approx. 756 mm. oxygen tension.....	Normal	Normal	Heliotropism lost, otherwise normal.

B. Observed 18 Hours after Return to Sea Water.

Isotonic NaCl at atmospheric oxygen tension.....	1/2 larvæ show bendings	1/50 larvæ show bendings	2/50 larvæ show bendings
Isotonic NaCl at approx. 756 mm. oxygen tension...	1/50 larvæ show bendings	No motion	No motion
Sea water at approx. 756 mm.....	Normal	Normal	Some heliotropic, some not heliotropic, all otherwise normal.

The increase in toxicity caused by the excessive oxygen tension is very marked.

The writer wishes to express his thanks to Professor R. S. Lillie under whose direction the problem was undertaken.

Conclusions.

1. The removal of most of the oxygen from the solution markedly diminishes the toxicity of sodium chloride isotonic to sea water to *Arenicola* larvæ.

2. Slight increases in oxygen tension also markedly diminish the toxicity of isotonic sodium chloride.

3. Saturation with oxygen markedly increases the toxicity of this solution.

4. At a tension of approximately 275 mm. the toxic and antitoxic effects balance.